Interaction of Dietary Vitamin E with *Eimeria maxima* Infections in Chickens

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ABSTRACT In two trials, broiler chickens, processed similarly to those placed in commercial operation, were fed, from 1 d of age, a range (13 to 200 ppm) of DL-α-tocopheryl acetate (VE-AC) levels, and the effects on the pathology of *Eimeria maxima* infections were assessed at 6 d postinoculation (PI). In Trial 1, dietary levels of VE-AC had little significant effect on variables characterizing pathology except for the number of oocysts shed, which was significantly increased in chicks treated with higher VE-AC levels. The infection was judged to be mild based on moderate lesion scores (2.2 ± 0.2), lack of significant effects on weight gain (7 ± 1.6% decrease), moderate reduction in plasma carotenoids (21 ± 2%) and small increases in plasma NO_2^-+NO_3^- (141 ± 12%). In uninfected and infected chickens, plasma α-tocopherol (AT) increased with dietary levels of VE-AC; however, *E. maxima* infection caused a fairly constant decrease in AT of 35.3 ± 3.2% across these levels. Plasma γ-tocopherol (GT) levels were unaffected by dietary VE-AC or *E. maxima* infection. In Trial 2, pathology, again, was relatively unaffected by dietary VE-AC level. The infection was judged to be severe based on lesion scores (3.5 ± 0.1), reduction in weight gain (30.7 ± 3%), plasma carotenoids (72.4 ± 1.5%), uric acid (16.3 ± 3.4), albumin (37.8 ± 2.8%), large increases (261 ± 8%) in plasma NO_2^-+NO_3^-, and high numbers of oocysts shed per chick ($4.12 \pm 0.4 \times 10^7$). Plasma AT again increased with increasing dietary VE-AC levels in uninfected and infected chicks, but the mean decrease across VE-AC levels caused by *E. maxima* infection was 73.14 ± 3.3%. GT levels were erratic and unrelated to dietary VE-AC or infection. Thus, in processed broiler chickens, high dietary VE-AC did not prevent or lessen the pathology caused by mild or severe infections with *E. maxima*. The main effect of *E. maxima* infection appeared to be reduction in plasma AT levels. We postulate that this reduction may be due to malabsorption of AT, which results from physical damage to the absorptive mucosa, reduction in esterases required to hydrolyze the VE-AC, and a generalized lipid malabsorption, preventing movement of the free AT to circulating blood and infected tissues.

(*Key words*: avian coccidiosis, α-tocopherol, γ-tocopherol, antioxidant, malabsorption)

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INTRODUCTION

Vitamin E (D-α-tocopherol; AT), is a multifunctional nutrient essential for normal growth and development of chickens (review by Ames, 1956). To afford increased stability, AT is generally added as DL-α-tocopheryl acetate (VE-AC) to commercial poultry feeds at levels from 17 mg/kg (Erf et al., 1998) to 48 mg/kg (McIlroy, 1996). After ingestion, VE-AC is hydrolyzed and is absorbed through the intestinal epithelium in its unesterified form. It is readily incorporated into cellular membranes, where it promotes integrity by functioning as an antioxidant, protecting against free radical oxidative processes (Tappel, 1972). AT can also function as an immunomodulator in chickens (Gore and Qureshi, 1997; Erf et al., 1998; Boa-Amponsem et al., 2000), in which high dietary supplements have been reported to boost humoral responses to *Escherichia coli* infection (Tengerdy and Brown, 1977; Nockles, 1988; Macklin et al., 2000), infectious bursal disease (McIlroy et al., 1993), and Newcastle disease (Franchini et al., 1986).

Effects of dietary AT supplementation on avian coccidiosis have been sparsely investigated. In one study (Colnago et al., 1984), supplements of 100 IU/kg AT increased body weight gains and decreased lesion scores after *Eimeria tenella* challenge infection of naive and homologously immunized chickens. However, results were inconsistent within a series of experiments. *Eimeria tenella* is only one of seven species that infect chickens. Each species infects

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Abbreviation Key: AT = α-tocopherol, GT = γ tocopherol, PI = postinoculation, VE-AC = DL-α-tocopheryl acetate.

a unique area of the digestive tract and elicits unique pathological effects.

For example, *Eimeria maxima*, a parasite that develops in the mid-small intestine, is considered to be the most immunogenic of the coccidia that infect chickens (Rose and Long, 1962). The immune response of the chick host includes generation of superoxide and nitric oxide free radicals at infection sites (Allen, 1997). These reactants possibly contribute significantly to the pathology of *E. maxima* infections, characterized by bloated midintestine, watery diarrhea, mucosal petichiation, orange casts of sloughed mucosa, significant reduction in weight gain, nutrient malabsorption, and decreased carotenoid pigmentation (which could be caused by malabsorption and carotenoid oxidation). Because AT is such a potent antioxidant, we hypothesized that supplementing the diet with high amounts of VE-AC might counteract the pathological effects of infection. We report here the results of two experiments designed to test this hypothesis.

MATERIALS AND METHODS

Animals and Housing

Day-old, male, Ross-based roaster-line chicks were obtained from a local Maryland hatchery. These chicks had been processed for commercial growout. That is, they received Marek's vaccine in ovo and live vaccination for Newcastle disease and infectious bronchitis. They were raised in Brower[2] brooders, 20 chicks per brooder, for 2 or 3 wk. During these periods, the temperatures of the brooders were gradually decreased from 37 to 27 C. The chickens were then wing-banded, weighed, divided into experimental groups, and placed in suspended cages, three to five chicks per cage, in rooms maintained between 25 and 28 C with continuous lighting. Chickens were provided feed and water ad libitum.

Feed and Supplements

A mash[3]-based diet was formulated from natural ingredients to provide a minimum of VE-AC (Table 1). Experimental diets were prepared by adding the following ingredients to each: 0.25% vitamin premix (Q5807150)[4] and varying levels of vitamin E premix (Rovimix E 20)[4] (Table 2).

Parasites

Two strains of *Eimeria maxima* were used: Strain ESS, purified from an isolate obtained from commercial poultry producers on Maryland's Eastern Shore, and Guelph, a Canadian strain.[5] These strains were maintained in the

[2]Brower Inc., Houghton, IA 52631.
[3]Formula 2630, Southern States Cooperative, Richmond VA 23260.
[4]Roche Vitamins, Parsippany, NJ 07054.
[5]This strain of *E. maxima* was obtained from J. Barta, U. Guelph, Guelph, Ontario.

TABLE 1. Composition of base mash for experimental diets[1]

Ingredients	g/kg
Added	
Wheat middlings	42.5
Corn meal	462
Brewers grains	50
Fat-feed grade	50
48% soybean meal	324
Copper sulfate	0.5
Ground limestone	5.8
Dicalcium phosphate	9
Salt (plain)	3.9
Trace mineral premix 434	0.5
Choline chloride, 70% liq.	0.5
DL-methionine	0.5
Mold-guard	0.5
0.06% selenium premix	0.3
60% Agway special[2]	50
Analyzed	g/kg mash
Crude protein	239.97
Crude fat	77.51
Crude fiber	28.16
Poultry ME, kcal/kg	3118
Calcium	8.5
Total phosphorus	7
Available phosphorus	4.2
Sodium	2
Chloride	2.9
Potassium	8.9
Iron	0.238

[1]This diet provided the following trace minerals (mg/kg mash): copper, 147; zinc, 137.4; manganese, 78.6; cobalt, 0.078; selenium, 0.349. Provides the following amino acids (g/kg mash): lysine, 13.63; methionine, 4.5; methionine+cystine, 8.8; arginine, 16.79; tryptophane, 3.18; histidine, 6.00; threonine, 9.51; isoleucine. 10.730. Provides the following fat-soluble vitamins per kg mash: vitamin A, 1.526 KIU; vitamin E, 13.76 mg; vitamin K-menadione, 0.093 mg. Provides the following water-soluble vitamins per kg mash: vitamin B_{12}, 0.842 μg; biotin, 0.198 mg; choline, 1.596 g; folic acid, 0.424 mg; niacin, 26.0 mg; pantothenic acid, 8.546 mg; pyridoxine, 0.292 mg; riboflavin, 1.835 mg; thiamin, 3.431 mg. (Southern States Cooperative, Richmond , VA 23260).

[2]Meat meal.

TABLE 2. Compositions of experimental diets

Diet vitamin E, ppm	Base mash	Base vitamin mix[1]	Vitamin E premix
	(g/kg diet)		
Trial 1			
Analyzed level			
13.2[3]	1,000	2.5	0
27.4	997	2.5	0.27
39.8	997	2.5	0.84
76	996	2.5	1.97
153	993	2.5	4.28
Trial 2			
Calculated level			
13.2[3]	1,000	2.5	0
25	997	2.5	0.4
50	996	2.5	1.23
100	995	2.5	2.9
200	991	2.5	6.2

[1]Provided fat-soluble vitamins (per kg diet): vitamin A, 9,912 IU, vitamin D3, 2,754 IU. Provides water-soluble vitamins (in mg/kg diet):vitamin B_{12}, 0.02; riboflavin, 6.6; niacin, 44.0; pantothenic acid, 11.0; menadione, 1.98; folic acid, 1.32; biotin, 0.11; thiamin (B1), 1.98; pyridoxine, 3.98; choline, 495.5.

[2]Contained 0.044 mg vitamin E (DL-α tocopheryl acetate) per gram of premix.

[3]Base mash plus base vitamin mix analyzed to contain 13.2 ppm AT.

laboratory by periodic passage through chickens. Oocysts of the isolates were stored at 4 C in 2.5% $K_2Cr_2O_7$. For use, the $K_2Cr_2O_7$ was washed out with tap water and by repeated centrifugation, and the concentrations were adjusted to the desired inoculum dose per chick in 1 mL of water. Effective infection doses were determined through pretrial titrations in age-matched chickens.

Plasma Analyses

Total carotenoids were extracted with acetone and quantified spectrophotometrically (Allen et al., 1996). Other analyses were adapted to a 96-well plate format. Uric acid was determined colorimetrically through reaction with uricase, peroxidase, and aminoantipyrene using a commercially available kit (Kit 684-25).[6] The reaction mixture per well included 10 μL plasma, standard or water, and 200 μL reagent.[4] Plates were read at 490 nm (peak) and 650 nm (baseline) 10 min after addition of reagent, and the differences in optical densities were measured. Plasma concentrations (m*M*) were determined from a standard uric acid series (0.028 to 0.9 m*M*). Albumin was determined in diluted plasma (1:10 with water) by binding of bromocresol green (dye binding reagent).[6] Reaction mixtures per well included 25 μL sample, standard or water, plus 200 μL of bromocresol green reagent. Plates were read at 650 nm, and the plasma concentrations (m*M*) determined from a standard series of bovine serum albumin (0.008 to 0.045 m*M*). Assayed normal human serum (Accutrol)[6] was used as a control for these latter two analyses. Levels of NO_2^-+NO_3^- in clear plasma filtrates[7] (Allen, 1997) were determined with the Griess reaction (Verdon et al., 1995). Tocopherols in plasma were determined by HPLC with modifications of previously described methods (Shearer, 1986; Aebischer et al., 1999). Briefly, 100 μL of plasma was mixed with 90 μL of ethanol and 10 μL of internal standard (α-tocopherol acetate, 1 mg/mL) and then extracted with 200 μL n-hexane. The hexane layer was dried under vacuum. The residue was dissolved in 100 μL of ethanol, and 20 μL was injected on a Primesphere[8] 5 steel column (C-18 250 $\times$ 4.6; 5 μ). The mobile phase was 93% methanol and 7% dichloroethane, and the flow rate was 1.5 mL/min. Tocopherols were detected with a photo diode array detector[9]set at 292 nm. Retention times on the column were calibrated with known tocopherols,[10] and the AT in the plasma samples was quantified using the internal standard.

Experimental Protocols

Two trials were conducted. In both trials, chicks were randomly assigned to five experimental diet treatments at 1 d of age and were maintained on those treatments throughout the experiments. At the time of infection, chicks within each diet treatment were weighed individually and were then divided into weight-matched (Gardiner and Wehr, 1950) experimental groups that were infected or uninfected.

Additionally, chicks from each diet treatment were set aside for quantification of oocyst shedding, and their feces were collected from Days 5 through 8 postinoculation (PI). Feces were homogenized in water, duplicate aliquots serially diluted, and duplicate counts made on final 1:6 dilutions in 2.5 *M* sucrose using McMasters-type chambers (Conway and McKenzie, 1991). The effects of infection on the chickens in the experimental groups were assessed at 6 d PI, at which time birds were again individually weighed, and individual weight gains determined. Chicks were then bled (heart puncture, with EDTA as anticoagulant), killed by cervical dislocation, and scored for midintestinal lesions (Johnson and Reid, 1970). Feed consumption per cage of chicks was measured over the 6-d infection periods, and feed conversions for those periods were determined on the basis of the sum of the individual chick weight gains in each cage.

Trial 1. Two hundred 1-d-old chicks were randomly assigned to five experimental treatments (40 chicks per diet) having the following analyzed levels of VE-AC: 13.2, 27, 36, 76, and 153 mg/kg. The chicks were maintained on those treatments throughout the experiment. At 22 d of age, chicks within each diet treatment were assigned to weight-matched control or infected groups. For performance determinations, each diet treatment consisted of 15 control and 15 infected chicks (three cages of five chicks). For oocyst output determinations, the remaining chicks on each dietary level were assigned to three cages per diet (up to three chicks per cage). Chicks in infected groups were each inoculated with 175,000 sporulated oocysts / mL of lab Strain ESS.

Trial 2. One hundred forty-five 1-d-old chicks were assigned to five experimental diets with the following VE-AC levels: the lowest based upon analysis, 13.2, and calculated levels 25, 50, 100, and 200 mg/kg. Chicks were maintained on those diets throughout the experiment. At 14 d of age, chicks within each diet treatment were assigned to weight-matched infected and control groups of 10 chicks each (two cages of five chicks). For oocyst output determinations the remaining chicks in each diet level were assigned to three cages per diet (three chicks per cage). Chicks in infected groups were each inoculated with 40,000 sporulated oocysts of the Guelph strain.

Statistics

Data were statistically analyzed using the general linear models procedure of SAS (1990). Differences among groups were determined using Duncan's multiple-range test. For plasma analyses, weight gains, and lesion scores, the experimental unit was considered an individual chicken. Oocyst shedding and feed conversion analyses were based on cage means.

[6]Sigma Chemical Co., St Louis, MO 63195.
[7]Centricon 30 spin filters, Millipore Corporation, Beverly, MA 01915.
[8]Phenomenex, Torrance, CA 90501.
[9]Waters, Inc., Milford, MA 01757.
[10] D-a-tocopherol (T3251), D-γ-tocopherol (T1782), D-α-tocopherol acetate (T3001), Sigma Chemical Co., St. Louis, MO 01915.

TABLE 3. Trial 1, pathological effects of infection with *Eimeria maxima* at 6 d postinoculation

Variable	Dietary VE-AC[1] (ppm)	Uninfected	Infected
Weight gain (g)	13.2	405 ± 8^{ab}	395 ± 15^{abc}
	27.4	406 ± 5^{ab}	378 ± 10^{bc}
	39.8	435 ± 8^{a}	401 ± 12^{ab}
	76	402 ± 10^{ab}	353 ± 22^{c}
	153	300 ± 15^{ab}	378 ± 24^{bc}
Feed conversion ratio	13.2	1.63 ± 0.02^{b}	1.65 ± 0.04^{b}
(g feed/g gain)	27.4	1.65 ± 0.06^{b}	1.68 ± 0.05^{ab}
	39.8	1.61 ± 0.03^{b}	1.68 ± 0.02^{ab}
	76	1.63 ± 0.06^{b}	1.82 ± 0.05^{a}
	153	1.65 ± 0.04^{b}	1.76 ± 0.06^{ab}
Lesion score	13.2	NA	2.80 ± 0.30^{a}
	27.4		2.40 ± 0.21^{ab}
	39.8		2.13 ± 0.26^{ab}
	76		2.07 ± 0.29^{ab}
	153		1.87 ± 0.27^{b}
Oocyst shed	13.2	NA	2.83 ± 0.28^{b}
	27.4		1.39 ± 0.31^{c}
(oocysts/chick × 10^6)	39.8		2.14 ± 0.28^{bc}
	76		3.89 ± 0.29^{a}
	153		4.52 ± 0.19^{a}

[a-c]Values are means ± SEM. Within a variable, means with no common superscripts are significantly different ($P < 0.05$).

[1]DL-α-tocopheryl acetate.

RESULTS

Trial 1

There were no significant differences among mean body weights of chicks in the five experimental dietary VE-AC treatments at the start of infections. Increasing levels of dietary VE-AC had no effect on weight gains or feed conversions of uninfected chickens during 6 d of infection (Table 3). In spite of the large inoculation doses, the resulting infections were mild. Although the 6-d PI weight gains were generally reduced by infection, only the mean gain of chicks in the 76 ppm diet treatment was significantly decreased with respect to controls (Table 3). This group was also the only one to show a significant increase in feed conversion relative to uninfected controls (Table 3).

Lesion scores gradually decreased with increasing dietary VE-AC level, but the only significant difference was seen between the 0 and 153 ppm VE-AC levels (Table 4). The pattern of oocyst shedding with respect to dietary VE-AC was erratic and significantly decreased from chicks on 27.4 ppm and significantly increased from chicks on 76 and 153 ppm (Table 3).

In uninfected chicks, plasma AT increased significantly with increasing dietary VE-AC. On the other hand, plasma GT levels tended to be reduced with increasing levels of dietary VE-AC being significantly lower at 76 ppm. Plasma carotenoids remained relatively constant as did plasma NO_2^-+NO_3^-, which was significantly decreased only in the 15 ppm group (Table 4). In infected chicks, plasma AT also increased significantly with increasing dietary VE-AC. However, levels were always numerically lower than controls and significantly lower in the 76 and 153 ppm infected groups (Table 4). Infection with *E. maxima* caused a percentage decrease in plasma AT of 35.3 ± 3.2 over the range of dietary VE treatments. Plasma GT levels were not significantly decreased by *E. maxima* infection or by the dietary level of VE-AC. Plasma carotenoids were significantly decreased (35.3 ± 3.22%) by *E. maxima* infection over all levels of dietary VE-AC (Table 4). Infection caused small, but significant, increases in plasma NO_2^-+NO_3^- in all groups except those fed 13.2 ppm VE-AC (Table 4).

Trial 2

At inoculation with *E. maxima* oocysts, chicks in all experimental groups had developed rales, suggesting a breakthrough of infectious bronchitis. Increasing dietary levels of VE-AC had no significant effect on weight gain at 6 d PI in uninfected control chicks (Table 5). Infection with the Guelph strain of *E. maxima* caused significant decreases in weight gain in chicks fed all levels of VE-AC. Additionally, infected chicks consuming 100 and 200 ppm VE-AC had significantly lower weight gains than those consuming 13 ppm VE-AC. Infection also caused numerically increased feed conversion ratios except for those chicks fed 100 ppm VE-AC (Table 5). Lesion scores, which were higher in Trial 2 than in Trial 1, were not affected by dietary levels of VE-AC. The numbers of oocysts shed were significantly higher from the group fed 25 ppm as compared to groups fed all the other levels of VE-AC (Table 5).

Plasma AT levels increased with increasing dietary VE-AC in uninfected and infected chicks (Table 6). However, levels of AT were depressed in control chicks, particularly in those fed 100 and 200 ppm VE-AC compared to levels observed in Trial 1. Infection with *E. maxima* caused a mean decrease in plasma AT of 73.14 ± 3.3% over the

TABLE 4. Trial 1, effect of *Eimeria maxima* infection of plasma analytes

Analyte	Dietary VE-AC[1] (ppm)	Uninfected	Infected
Plasma α-tocopherol (μg/mL)	13.2	2.67 ± 0.21^{d}	1.67 ± 0.30^{d}
	27.4	3.76 ± 0.27^{cd}	2.56 ± 0.15^{d}
	39.8	6.16 ± 0.26^{c}	4.64 ± 0.25^{cd}
	76	9.66 ± 0.21^{b}	5.95 ± 0.13^{c}
	153	20.54 ± 0.27^{a}	11.57 ± 0.14^{b}
Plasma γ-tocopherol (μg/mL)	13.2	0.42 ± 0.03^{a}	0.30 ± 0.05^{ab}
	27.4	0.27 ± 0.04^{ab}	0.15 ± 0.05^{b}
	39.8	0.26 ± 0.05^{ab}	0.25 ± 0.10^{ab}
	76	0.21 ± 0.07^{b}	0.13 ± 0.04^{b}
	153	0.27 ± 0.05^{ab}	0.14 ± 0.10^{b}
Plasma carotenoids (μg/mL)	13.2	4.05 ± 0.14^{ab}	3.34 ± 0.22^{c}
	27.4	4.01 ± 0.20^{ab}	3.10 ± 0.14^{c}
	39.8	4.26 ± 0.14^{a}	3.56 ± 0.21^{bc}
	76	4.23 ± 0.25^{a}	3.34 ± 0.22^{c}
	153	4.18 ± 0.15^{a}	3.04 ± 0.28^{c}
Plasma NO_2^-+NO_2^- (m*M*)	13.2	10.58 ± 0.49^{bc}	12.58 ± 0.42^{ab}
	27.4	6.71 ± 0.56^{d}	12.32 ± 0.49^{ab}
	39.8	9.25 ± 0.72^{c}	11.69 ± 0.53^{b}
	76	9.19 ± 0.69^{c}	11.79 ± 1.06^{b}
	153	9.34 ± 0.46^{c}	13.92 ± 1.04^{a}

[a-d]Values are means ± SEM. Within a variable, means with no common superscript are significantly different ($P < 0.05$).

[1]DL-α-tocopheryl acetate.

range of dietary VE-AC levels. However, infected chicks fed 200 ppm VE-AC had plasma AT levels significantly higher than those fed 13 to 50 ppm VE-AC. Plasma levels of GT were generally not statistically affected by *E. maxima* infection. However, the GT levels in infected chicks fed 25 ppm VE-AC were significantly higher than that of the respective control chicks (Table 6). Infection caused a mean decrease in plasma carotenoids of 72.4 ± 1.5% over all levels of dietary VE-AC and a mean increase of 261 ± 8% in plasma NO_2^-+NO_3^- (Table 6). Increased dietary levels of VE-AC did not significantly change plasma levels of uric acid or albumin in uninfected control chicks. However, infection with *E. maxima* decreased plasma uric acid an average 16.4 ± 3.4% and albumin 37.8 ± 2.8% (Table 6).

DISCUSSION

The chickens used in these experiments were from a hatchery serving a commercial operation, and they all

TABLE 5. Trial 2, pathological effects of infection with *Eimeria maxima* at 6 d postinoculation

Variable	Dietary VE-AC[1] (ppm)	Uninfected	Infected
Weight gain (g)	13	359 ± 15^{a}	291 ± 28^{bc}
	25	354 ± 14^{a}	248 ± 25^{cd}
	50	371 ± 16^{a}	239 ± 20^{cd}
	100	329 ± 17^{ab}	225 ± 12^{d}
	200	378 ± 10^{a}	240 ± 17^{d}
Feed conversion ratio (g feed/g gain)	13	1.76 ± 0.02^{ab}	1.98 ± 0.18^{ab}
	25	1.86 ± 0.08^{ab}	2.07 ± 0.23^{ab}
	50	1.70 ± 0.01^{b}	2.30 ± 0.36^{a}
	100	2.14 ± 0.18^{ab}	1.99 ± 0.08^{ab}
	200	1.66 ± 0.02^{b}	1.99 ± 0.12^{ab}
Lesion score	13	NA	3.3 ± 0.2^{a}
	25		3.4 ± 0.2^{a}
	50		3.8 ± 0.1^{a}
	100		3.4 ± 0.3^{a}
	200		3.6 ± 0.4^{a}
Oocysts shed (Oocysts/chick × 10^7)	13	NA	3.85 ± 0.49^{b}
	25		5.61 ± 0.52^{a}
	50		3.78 ± 0.84^{b}
	100		3.62 ± 0.36^{b}
	200		3.85 ± 0.32^{b}

[a-d]Values and means ± SEM. Within a variable means with no common superscript differ significantly ($P < 0.05$).

[1]DL-α-tocopheryl acetate.

TABLE 6. Trial 2, effects of infection on plasma analytes

Analyte	Dietary VE-AC[1] (ppm)	Uninfected	Infected
Plasma α-tocopherol (μg/mL)	13	2.3 ± 0.25^{cde}	0.51 ± 0.14^{e}
	25	3.89 ± 0.38^{bcd}	0.80 ± 0.16^{e}
	50	6.18 ± 0.67^{b}	1.52 ± 0.10^{de}
	100	10.08 ± 0.80^{a}	2.79 ± 0.53^{cde}
	200	11.81 ± 0.28^{a}	4.63 ± 0.59^{bc}
Plasma γ-tocopherol (μg/mL)	13	0.18 ± 0.06^{bc}	0.34 ± 0.18^{abc}
	25	0.24 ± 0.10^{bc}	0.77 ± 0.30^{a}
	50	0.21 ± 0.05^{bc}	0.58 ± 0.24^{ab}
	100	0.18 ± 0.06^{bc}	0.02 ± 0.02^{c}
	200	0.12 ± 0.05^{bc}	0.04 ± 0.03^{c}
Plasma carotenoids (μ/mL)	13	3.8 ± 0.3^{abc}	1.1 ± 0.2^{d}
	25	4.1 ± 0.4^{a}	0.9 ± 0.1^{d}
	50	3.3 ± 0.2^{c}	1.0 ± 0.1^{d}
	100	4.0 ± 0.3^{ab}	1.1 ± 0.2^{d}
	200	3.4 ± 0.3^{bc}	1.0 ± 0.1^{d}
Plasma NO_2^-+ NO_3^- (μM)	13	10.90 ± 1.30^{b}	25.96 ± 2.84^{a}
	25	11.30 ± 1.28^{b}	28.10 ± 2.77^{a}
	50	10.32 ± 1.11^{b}	29.78 ± 2.80^{a}
	100	10.03 ± 0.59^{b}	26.71 ± 3.82^{a}
	200	11.03 ± 0.62^{b}	29.17 ± 3.34^{a}
Uric acid (mM)	13	0.461 ± 0.04^{abc}	0.410 ± 0.03^{c}
	25	0.538 ± 0.05^{a}	0.438 ± 0.04^{c}
	50	0.475 ± 0.03^{abc}	0.444 ± 0.03^{c}
	100	0.568 ± 0.04^{a}	0.421 ± 0.03^{c}
	200	0.490 ± 0.02^{bc}	0.394 ± 0.04^{c}
Albumin (mM)	13	0.338 ± 0.018^{a}	0.244 ± 0.014^{b}
	25	0.333 ± 0.020^{a}	0.197 ± 0.023^{b}
	50	0.364 ± 0.019^{a}	0.205 ± 0.018^{b}
	100	0.360 ± 0.016^{a}	0.212 ± 0.026^{b}
	200	0.328 ± 0.009^{a}	0.212 ± 0.024^{b}

$^{a\text{-}e}$Values are means ± SEM. Within a variable, means with no common superscript are significantly different ($P < 0.05$).

[1]DL-α-tocopheryl acetate.

received Marek's vaccine in ovo, and live vaccines, via spray cabinet, for Newcastle disease virus and infectious bronchitis virus. No adverse effects of this processing were observed with chicks in Trial 1, but chicks in Trial 2 developed rales, suggesting a break of infectious bronchitis.

In both trials, increased dietary levels of VE-AC were associated with increased plasma AT levels, but they had no significant effects on weight gain or feed conversion in uninfected chicks, which is consistent with reports of others (Bottje et al., 1997; Jensen et al., 1999). *Eimeria maxima* infections generate superoxide and NO free radicals (Allen, 1997), effectors of oxidative stress. Because AT is such a potent antioxidant, it was hoped that feeding supplements high in VE-AC would result in some protection, particularly in terms of lesion scores. However, supplementation of feed with up to 200 ppm VE-AC had no consistent beneficial effect on the pathology caused by mild or severe infection with *E. maxima*. Plasma uric acid and albumin are considered the principal antioxidants in plasma based on mass and activity (Wayner et al., 1987), as they are able to trap peroxyl-free radicals and, thus, spare or regenerate AT. Both of these components decreased during the infection in Trial 2. Levels were not influenced by dietary VE-AC in uninfected or infected chickens. As measured at Day 6 PI, during the acute phase of infection, reductions in uric acid probably reflect anorexia and mabsorption occurring at that time, whereas reduction in albumin may reflect leakage of this protein into the intestinal lumen because of the damaged mucosa. These reduced levels could thus additionally contribute indirectly to a state of oxidative stress during *E. maxima* infection.

The principal finding in this study is that during a mild (Trial 1) or a severe (Trial 2) infection with *E. maxima*, plasma AT is reduced by a relatively constant percentage, (35.5% or 73%, respectively) regardless of dietary VE-AC level. The percentages in plasma AT reduction are primarily influenced by the severity of infection. They also parallel the percentages of reduction in plasma carotenoids (21 and 72%, respectively), suggesting a common mechanism.

In humans, lipid malabsorption causes decreases in plasma carotenoids (Bottaro et al., 1992) and is a common cause of decreased plasma AT, leading to deficiency syndromes (Sokol, 1988; Dimitrov et al., 1996; Tanyel and Mancano, 1997). In chickens, many diverse conditions are associated with lipid malabsoption, including mycotoxicoses (Coffin and Combs, 1981; Osborne and Hamilton, 1981), intestinal viral infections (pale-bird syndrome and Newcastle's disease; Tyczkowski et al., 1991b), and, most importantly, coccidiosis (Ruff, 1978; Turk, 1978).

Lipid malabsorption during coccidiosis has been documented primarily by measuring decreased plasma carot-

enoids (as lutein equivalents) (Yvore and Mainguy, 1972; Ruff et al., 1974; Tyczkowski et al., 1991a; Allen, 1992). Most of this work has been done in chickens infected with *E. acervulina*, a parasite that principally infects the chick duodenum but that also affects the functioning of the jejunal mucosa (Allen, 1984). However, infection with *E. maxima*, which principally parasitizes the jejunal mucosa, causes very similar pathologies of reduced weight gain and nutrient malabsorption (Ruff and Wilkins, 1980) including carotenoid malabsorption (Tables 4 and 6). Additionally, infection with this parasite may cause some decrease in carotenoids through oxidative mechanisms (Allen, 1997).

Eimeria maxima infection may cause malabsorption of dietary supplements of VE-AC through several mechanisms. First, to be absorbed from the intestine, the VE-AC must be hydrolyzed in the lumen to the free AT by pancreatic carboxy ester hydrolases, the pH optimum of which is 8 (Lombardo et al., 1980) to 8.5 (Mathias et al., 1981). Coccidia infections (e.g., *E. acervulina*; Kouwenhoven and van der Horst, 1969, 1972) lower the pH of the intestinal lumen and, thus, can retard the action of the hydrolases.

Second, coccidia infections that affect the jejunal mucosa qualitatively and quantitatively change the absorption of lipids. Sharma and Fernando (1975) observed lipid droplets apparently trapped in villous epithelial cells parasitized with gamonts of *E. acervulina*. The portomicron plus very-low-density lipid fractions were barely visible in density gradient separations of plasma from chicks at 5 d PI with *E. acervulina* (Allen, 1988). These are the fractions primarily involved in transporting AT out of the intestine (Drevon, 1991; Cohn et al., 1992). Additionally all classes of plasma lipids are significantly reduced in a temporal pattern differing from that observed in chicks on feed withdrawal (Allen, 1988).

Third, qualitative changes occur in the absorptive surface, which is diminished due to sloughing and villous atrophy (Witlock and Ruff, 1977). The rate of mucosal cell turnover, which is relatively rapid, is further increased during coccidia infections (Pout, 1967; Fernando and McCraw, 1973; Michael, 1974; Witlock and Ruff, 1977) and the metabolic activities of this tissue change (Allen, 1984, 1987). All of these changes could adversely affect the absorption, transport, and incorporation of AT into mucosal membranes and reduce its effectiveness as an antioxidant or immune modulator during the time the parasite is developing in the mucosal epithelium. Although tissues would be loaded with AT during preinfection supplementation (Sheehy et al., 1991), malabsorption, as indexed by reduction in plasma carotenoids, reaches significant proportions as early as d 3 PI (Allen, unpublished data) requiring early mobilization of tissue stores that would then not be refilled during the completion of the parasite's life cycle. If lipid malabsorption is the fundamental reason why elevated dietary levels of VE-AC were ineffective in reducing pathologies associated with *E. maxima* infection in these present trials, then it may also explain the positive effects on body weights and lesion scores demonstrated by Colnago et al. (1984) in feeding VE-AC to *E. tenella*-infected chicks. *Eimeria tenella* infects the cecal mucosa and is not a major effector of lipid malabsorption.

In summary, providing high dietary levels of VE-AC did not afford any antioxidant protection to chickens infected with *E. maxima*. Plasma AT levels were reduced by infections at a constant percentage over ranges of dietary levels, and this reduction was dependent upon the severity of infection. The most reasonable explanation for these effects is that lipid malabsorption occurring during the infections prevented access of the dietary AT to the infected tissues.

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